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VARIATION IN THE NUMBER OF PHYLLOSPHERIC NITROGEN FIXERS AND OTHER MICROFLORA IN RELATION TO AGE OF THE PLANT

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ABSTRACT

Using dilution plate technique, the changing pattern of microbial population on the phylloplane of rice, wheat and jute plants were estimated with respect to age of the plants. It was noticed that nitrogen fixing, as well as other bacterial and fungal populations, were relatively low on the leaf surfaces at the seedling stage but the number gradually increased with the maturity of plants. A decline in population was noted during senescence of the plants. It was also found that the number of nitrogen fixers and other flora were greater in the rainy season crops than in the winter and summer ones.

INTRODUCTION

THE occurrence of microorganisms as regular inhabitants on the leaf surfaces of almost all plants is now well appreciated. The density and variance of such organisms depend on a number of factors. Climatic or environmental factors along with some basic characteristics of the host plant itself play a decisive role in the distribution of these microbes. Although several investigators have worked on the microbial settlers of various plants, including perennial trees, the age factor of the plants had hardly been considered. From arboreal plants mature leaves are generally used for microbial studies and the question of plant age does not arise, but for annuals or crop plants the age of the plant should be of prior consideration. Normally the leaves of seedlings are initially free of microbes and the earliest colonisers do not face any competition but may gain some profit from the supply of surface nutrients. Of the initial colonisers, some may immediately start growing while others may not flourish till they are adapted to this environment or till they get adequate substrate for their growth. Ruinen[1] and Shishiyama *et al.*[2], reported that the phylloplane microbes may utilise the cuticle by the activity of lipolytic enzymes produced by them. The cuticle thickness increases with aging of the leaves and it is obvious that cuticle utilisers can flourish only with aging of the leaves. McBride[3] reported that bacterial and yeast populations were low on young leaves but increased rapidly with increasing leaf age in larch and other plants, the number reaching approximately 2×10^6 organisms per gram of leaves with maturity. Sinha[4] demonstrated in *Capsicum annum*, *Solanum melongena*, *S. tuberosum* and *Lycopersicum esculentum* that older leaves bear much larger number of microbes than younger leaves. Sharma and Mukherjee[5] observed that on *Gossypium* and *Sesamum* the youngest leaves had the lowest number of bacteria whereas the oldest had the highest. It might be mentioned here, that with increasing age, the cuticular deposition as well as leaching are increased. Concomitant with these, the number of organisms are likely to increase. To understand when the plants are best colonised or to utilise the isolated phylloplane organisms as biofertilisers, it is extremely important to know the ideal age of the host plants when these intruders can best thrive. It may be highly rewarding if the beneficial organisms are sprayed at a time when potentiality of their growth on leaf surface is high. However, investigations directed towards this goal have never been conducted. This communication is meant to describe the variation in the number of nitrogen fixing bacteria and other microflora on the leaf surfaces of rice, jute and wheat of varying ages.

MATERIALS AND METHODS

Wheat (Cultivar Sonalika), jute (Cultivar JRO-632) and two cultivars of rice (Pusa 2-21 and Ratna) were grown in the agricultural farm of Calcutta University at Baruipur, 40 km south of the University Science College, where the laboratory work was done. Leaves were collected from these plants at different seasons. Individual healthy leaves free from insects and visible soil particles were selected from 15 days old plants. Generally, the second and third leaf, counted from the base of the plant (in jute) or from the tillers (wheat and rice) were removed at random and collected in separate sterile plastic bags. During the next sampling the 5th and 6th leaves were collected because most of the older leaves had reached the senescent stage. Samples were obtained at 15 days intervals from the seedling stage (15 days) to seed ripening and brought immediately to the laboratory for analysis. The total surface area of the leaves were measured using a planometer.

TABLE I

Incidence of nitrogen fixing bacterial population along with other organisms on the leaf surfaces of some crop plants of varying age

Age of the plant in days	Nitrogen fixing bacterial population				Other microbial population							
	10^3/Sq. cm of leaves				Bacteria 10^4/Sq. cm of leaves				Fungi 10^2/Sq. cm of leaves			
	Wh*	R_1*	R_2*	Jute	Wh*	R_1*	R_2*	Jute	Wh*	R_1*	R_2*	Jute
15	0·005	0·002	0·03	0·07	0·026	0·008	0·002	0·004	0·012	0·014	0·017	0·03
30	0·02	0·16	0·08	0·18	1·31	0·17	0·05	0·06	0·013	0·02	0·02	0·16
45	1·02	0·32	2·80	5·01	16·04	2·55	0·76	2·02	0·08	0·05	0·16	2·30
60	1·97	0·94	8·01	8·92	23·06	6·46	11·42	6·75	0·69	0·15	0·81	3·08
75	2·50	1·57	18·04	16·15	26·07	18·09	15·15	7·54	9·05	0·49	2·51	2·45
90	1·63	1·23	27·06	18·74	24·08	26·41	32·27	9·61	9·57	1·63	3·94	1·87
105	1·64	1·20	25·03	16·12	25·05	21·64	50·51	7·66	9·68	1·38	2·86	1·66
120			24·07	16·37		16·66	49·18	7·12				1·82

*Wh = Wheat, R_1 = Rice (Pusa 2–21), R_2 = Rice (Ratna).

Leaves of measured area were cut into small pieces and placed in 100 ml sterile Erlenmeyer flasks containing 20 ml sterilised distilled water together with 3 to 4 glass beads. The flasks were then fitted with rubber bungs and agitated for 20 minutes on a rotary shaker. Dilutions were made upto 10^{-3} fold with sterilised water. About 0·1 ml portions of each dilution were pipetted on petridishes containing 20 ml of solidified medium, either nutrient agar or potato dextrose agar or nitrogen-free Burk's agar, to find out the total number of bacteria, fungi and nitrogen fixers respectively. The suspension in each plate was subsequently spread with the help of a sterile spreader. For each dilution, five replicates were incubated at 30° C for 4–7 days, depending on the nature of growth.

RESULTS AND DISCUSSION

From an examination of Table I it is evident that at the seedling stage very few organisms were present on the leaf surface, the number gradually increased till the senescent stage of the plants, after which the number started dwindling. From wheat and one variety of rice (Pusa 2–21) maximum number of nitrogen fixers were recorded when the plants were 2½ months old, whereas from another variety of rice (Ratna) and jute the highest number was at the 3 months stage, the numbers subsequently decreasing. A declining trend of total bacterial and fungal population was also noted at the later stages of growth of these plants. In wheat and rice (Variety Ratna) this decline was not prominent. The population pattern in the three crop plants, even in the two varieties of rice, varied greatly in relation to their age. Maximum propagules were isolated from rice (Variety Ratna) phyllosphere. The other variety of rice (Pusa 2–21) also supported a good bacterial population while the wheat leaf surface contained the highest number of fungal propagules. The variation in number may be due to differential supply of nutrients on the leaf surfaces as is evident from the works of McBride and Hayes[6] and Ruinen[7].

Regarding the nutrition of phyllosphere organisms, Ruinen[8] claimed that cuticle is the source of nutrients whereas Diem[9] considered that pollen grains deposited on leaf surface are utilised by the phyllosphere population. If cuticle is considered as nutrient, then the mature leaves are better equipped to support the nutrition of these organipms. Similarly it has been reported that nutrients are more easily leached out of mature leaves (Arens[10] and Tukey *et al.*[11]). Therefore it is quite likely that adult leaves are better source of nutrients for phyllosphere organisms.

In comparison, the number of organisms reported from other leaves, much lower number of organisms were obtained. The findings in the present study are in conformity with the work of Clark and Paul[12] who reported that broad leaved plants like legumes and vegetables harbour more organisms than the narrow leaved plants like grasses. Here too much higher number of microbes were found on the jute leaf surface than on those of rice or wheat. Dickinsen *et al.*[13] observed progressive increase in the population of bacteria from May to September on *Lolium perenne* leaves. This has been correlated with the alteration in characteristics of leaves, nutrient sources and activity of the phylloplane microbes. McBride[3] also noted that development of phyllosphere microflora of larch and several other plant species had a distinct pattern which was apparently related to leaf age. He also noticed that bacterial and yeast populations were low on young leaves and increased rapidly with increasing leaf age to reach approximately 2×10^6 organisms per gram of leaves at the time of leaf fall. Stott[14] observed an increase in population of microbes on *Beta vulgaris* leaves with increasing age of the plants. Sharma and Mukherjee[5] observed that on *Sesamum* and *Gossypium* the youngest leaves had the lowest number of bacteria whereas the oldest had the highest. The number of organisms depended greatly on the time of sampling (Bessem[15]) and on the age of the leaves, increasing from a basic number of 10^5/ml in the top portion of leaves to 10^{10}/ml in the mature sheaths and decreasing again to less than 10^5/ml during senescence and death of the leaves.

Quantitative data on the population density are widely divergent and should be considered in relation to plant species and the environmental condition of the habitat. It may be due to the amount and nature of leachings from the leaves in different ecological conditions. Or, it may be the fact that there are some plants whose leaching capacities are very small. It has been ascertained by Tukey *et al.*[11] that the carbohydrate loss from leaves is directly correlated with the light intensity and wetting. It is true in our case because wheat is a winter crop whereas jute and rice are rainy season crops. Again Tukey *et al.*[11] reported higher amount of carbohydrate and soluble protein leakage from the leaves due to dew and rains while lower amount of loss is reported by Nye[16].

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